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PCT/SE99/00398

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

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Date of mailing (day/month/year)
14 December 2000 (14.12.00)

Applicant's or agent's file reference
54842-AKR

IMPORTANT NOTIFICATION

International application No.
PCT/SE99/00398

International filing date (day/month/year)
15 March 1999 (15.03.99)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

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2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

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3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
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Form PCT/IB/306 (March 1994)

003724233

Replaced by Amended

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

15

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
50437-54842		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/SE99/00398	15.03.1999	13.09.1998

International Patent Classification (IPC) or national classification and IPC7

C 12 N 15/87, C 07 K 19/00

Applicant

Karolinska Innovations AB et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12.04.2000	09.01.2001
Name and mailing address of the IPEA/SE	Authorized officer
Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. 08-667 72 88 Telex 17978 PATOREG-S	Patrick Andersson/EÖ Telephone No. 08-782 25 00

Form PCT/IPEA/409 (cover sheet) (January 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE99/00398

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages 1-29 , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the claims:
pages _____ , as originally filed
pages _____ , as amended (together with any statement) under article 19
pages _____ , filed with the demand
pages 1-3 , filed with the letter of 22.11.2000

☒ the drawings:
pages 1-6 , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☐ the sequence listing part of the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE99/00398

III. Non-establishment f opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 26-27

because:

☒ the said international application, or the said claims Nos. 26-27
relate to the following subject matter which does not require an international preliminary examination *(specify)*:

See PCT Rule 67.1.(iv).: Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____
are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos. _____

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE99/00398

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	6-7,10,12,14,21-22,24-25	YES
	Claims	1-5,8-9,11,13,15-20,23	NO
Inventive step (IS)	Claims	6-7,10,12,14,21-22,24-25	YES
	Claims	1-5,8-9,11,13,15-20,23	NO
Industrial applicability (IA)	Claims	1-25	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The claimed invention relates to a method of transferring and/or directing a nucleic acid of interest across a biological membrane. The method uses a synthetic transport entity for this purposes and comprises:
a) Providing a carrier molecule comprising the nucleic acid and a binding element (BE) target sequence;
b) Providing a complex by coupling at least one functional element (FE) to a BE;
c) Hybridising the BE of the complex to the BE of the target of the carrier; and
d) Contacting the transport entity with the biological membrane to transfer the nucleic acid across it. The invention also relates to a kit for performing the method, a recombinant cell and a transport entity and its uses.

The following documents are considered relevant:
D1) WO93/19768
D2) WO96/11205

D1 discloses a method for oligonucleotide delivery and gene therapy, one of the objects of the invention is to provide a composition for presenting a polynucleotide to a subcellular component comprising a subcellular-localisation component associated with the polynucleotide. In D1 the DNA-associating moiety i.e. BE can be covalently associated with a functional component e.g. a nuclear-localisation component see page 13 line 20-page 14 line 14. The object is to cross the cell wall and/or the nuclear membrane with for example a plasmid.

.../...

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

The document does not disclose the possibility to use an entity capable of membrane translocation as well as nuclear transport. No specific disclosure of PNA being the DNA-associating moiety is provided; however, the expression "PNA analogue" in the present claims includes complementary single stranded DNA suggested as a BE in D1 page 13 line 32-33, since PNA is a DNA analogue.
Thus, the invention according to claims 1-5, 8-9, 11, 13, 15-20, and 23 lacks novelty and consequently an inventive step.

D2 discloses a PNA linked to a sequence derived from SV 40 large T antigen protein, presumably an NLS. D2 represent the general state of the art of no particular relevance for the present claims.

The invention according to claims 6-7, 10, 12, 14, 21-22 and 24-25 is considered to be novel, industrially applicable and to involve an inventive step.

CLAIMS

1. A method of transferring a nucleic acid of interest across a biological membrane, and/or direction thereof to a specific location within or on a cell, by use of a synthetic transport entity; which comprises the steps of
   (a) providing a carrier molecule comprising the nucleic acid of interest and a binding element (BE) target sequence;
   (b) providing a complex by coupling at least one functional element (FE) to a binding element (BE);
   (c) hybridising the BE of said complex to the BE target of said carrier; and
   (d) contacting said transport entity with said biological membrane to provide for a transfer of the nucleic acid of interest across said membrane.
2. A method according to claim 1, wherein the BE is a peptide nucleic acid (PNA) or a derivative or an analogue thereof.
3. A method according to claim 1 or 2, wherein in step (b), a complex is provided, wherein said BE and FE(s) are separated by linker element(s).
4. A method according to any one of the preceding claims, wherein in step (a), the carrier provided is a plasmid or an oligonucleotide vector comprising said nucleic acid of interest and at least one BE target sequence.
5. A method according to any one of the preceding claims, wherein in step (a), a detectable marker element is also inserted in said carrier.
6. A method according to any one of the preceding claims, wherein the nucleic acid of interest is a gene encoding a peptide, a protein or an RNA.
7. A method according to any one of the preceding claims, wherein the biological membrane is a cell wall.
8. A method according to claim 7, wherein in step (b), an FE comprising an antennapedia peptide is provided in said complex, which enables said transfer of the nucleic acid of interest across the cell wall.
9. A method according to any one any one of claims 1-6, wherein the biological membrane is a nuclear membrane.

10. A method according to claim 9, wherein in step (b), an FE comprising a nuclear localization signal is provided in said complex, which enables said transfer of the nucleic acid of interest across a nuclear membrane.

11. A method according to any one of claims 1-6, wherein in step (b), an FE comprising a protein, such as an HIV protein, e.g. TAT, is provided in said complex, which enables both membrane translocation and nuclear transport of the nucleic acid of interest.

12. A kit comprising components for making a transport entity capable of for transferring a nucleic acid of interest across a biological membrane, and/or direction thereof to a specific location within or on a cell, which kit comprises a binding element (BE); a functional element (FE); an oligonucleotide comprising a target for said BE suitable for cloning in a desired plasmid containing said nucleic acid of interest; and optionally reagents suitable for such transfer and/or direction.

13. A kit according to claim 12, wherein the binding element is a PNA and the target is a PNA target sequence.

14. A kit according to claim 12 or 13, wherein the functional element (FE) is an antennapedia peptide.

15. A kit according to claim 12 or 13, wherein the functional element (FE) is a nuclear localisation signal (NLS), such as a SV40 large T antigen protein, or a fragment thereof exhibiting nuclear localizing signal properties.

16. A kit according to claim 12 or 13, wherein the functional element (FE) is a protein enabling both membrane translocation and nuclear transport.

17. A synthetic transport entity suitable for use in the method according to any one of claims 1-11, which is comprised of at least one functional element (FE), which is complexed to a binding element (BE), and a nucleic acid carrier, which comprises at least one BE target sequence and a nucleic acid of interest in a vector; said complex being hybridised to said carrier using the BE-BE target interaction.

18. A transport entity according to claim 17, wherein the BE is a peptide nucleic acid (PNA) or a derivative or an analogue thereof.

19. A transport entity according to claim 17 or 18, wherein said vector is a plasmid or an oligonucleotide.

20. A transport entity according to any one of claims 17-19, wherein the carrier includes a detectable marker element.

21. A transport entity according to any one of claims 17-20, wherein the nucleic acid of interest is a gene encoding a peptide, a protein or an RNA.

22. A transport entity according to any one of claims 17-21, wherein said BE and FE(s) are separated by linker element(s).

23. A transport entity according to any one of claims 17-22, which comprises more than one FE-BE-complex, each one of which is hybridised to a separate BE target sequence present on the same carrier.

24. A transport entity according to claim 23, wherein each FE-BE-complex comprises two or more FEs, preferably spaced by linkers.

25. A transport entity according to any one of claims 17-24, wherein the FE is an antennapedia peptide.

26. A transport entity according to any one of claims 17-24, wherein the FE is a nuclear localization signal (NLS), such as a SV40 large T antigen protein, or a fragment thereof exhibiting nuclear localizing signal properties.

27. A transport entity according to any one of claims 17-24, wherein the FE is a protein, such as an HIV protein, e.g. TAT, exhibiting properties enabling both membrane translocation and nuclear transport.

28. A recombinant cell comprising one or more genetic modification(s) provided by use of the method as defined in claim 1-11 or a transport entity as defined in claims 17-27.

29. Use of a transport entity according to any one of claims 17-27 or a cell according to claim 28 in gene therapy.

30. Use of a transport entity according to any one of claims 17-27 or a cell according to claim 29 in DNA-vaccination.